Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 3, 5-10, 13, 16, 17, 19, 26, 28, 29, 34-38 and 40-44 are pending in the application, with claims 1, 37, and 38 being the independent claims. Claims 1, 9 and 37 were amended with the Examiner's suggestions in mind. Support for the amendment can be found, *inter alia*, at page 4, lines 3-14; at page 18, line 26 to page 19, line 25; and at page 21, line 21 to page 22, line 14. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Information Disclosure Statement

Applicant again notes that the Examiner did not initial document AP1 cited in the First Supplemental Information Disclosure Statement filed on August 28, 2000. (*See* copy of initialed 1449 Form attached to Paper No. 15.) Applicant respectfully requests that the Examiner initial the document cited on the 1449 Form and return a copy of the initialed 1449 Form to Applicant, or indicate why the document was not considered.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1, 3, 5-10, 13, 16, 17, 19, 26, 28, 29, and 34-37 under 35 U.S.C. § 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. (Paper No. 27, at page 2.) Applicant respectfully disagrees.

Applicant submits that it is clear what he considers the "3'-5' exonuclease domain" and the "5'-3' exonuclease domain" to be. The 3'-5' and 5'-3' exonuclease domains correspond to *Tma* DNA polymerase sequences that encode portions of the *Tma* DNA polymerase exhibiting 3'-5' exonuclease activity and 5'-3' exonuclease activity, respectively. *See also* U.S. Patent No. 5,374,553 (which is incorporated by reference as indicated at page 18, lines 23-25 of the specification), which contains *Tma* DNA polymerase amino acid sequence. Since the meets and bounds of the claims are clear, withdrawal of this rejection is respectfully requested.

In addition, in an effort to advance prosecution, Applicant has amended claim 1 to read a "*Thermotoga maritima (Tma)* DNA polymerase which is modified at least two ways" and claim 9 to read "[t]he mutant *Tma* polymerase of claim 1, wherein the modifications are . . . " The claims as amended distinctly claim the subject matter which Applicant regards as the invention. Thus, Applicant respectfully requests that the Examiner withdraw the rejections under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner maintained the rejection of claims 1, 3, 5-10, 13, 16, 17, 19, 26, 28, 29, and 34-37 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter

which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. (Paper No. 27, at page 4.) The Examiner alleged that

> [t]he specification only provides a **Tne** DNA polymerase mutant consisting of a combination of the following mutations: those mutants having reduced 3'-5' exonuclease activity consisting of Asp^{323} converted to Ala^{323}, those mutants having reduced discriminatory behavior against a dideoxynucleotides [sic], consisting of Phe^{730} converted to Tyr^{730} and those mutants having reduced 5' 3' [sic] exonuclease activity consisting of the deletion of 219 amino terminal amino acids of Tne DNA polymerase and Asp^{8} converted to Ala^{8} or Asp^{137} converted to Ala^{137}(Examples 11-13 and 16). There is no disclosure of any particular structure to function/activity relationship in the claimed genus. The specification also fails to describe additional representative species of these DNA Polymerases by any identifying structural characteristics or properties other than having reduced 3'-5' exonuclease activity of the polymerase, reduced 5'-3' exonuclease activity or reduced discriminatory behavior against dideoxynucleotides for which no predictability of structure is apparent.

Id. at pages 4-5 (emphasis in original). Applicant respectfully disagrees.

The test for the written description requirement is whether one skilled in the art could reasonably conclude that the inventor has possession of the claimed invention in the specification as filed. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 U.S.P.Q.2d 1111, 1116 (Fed. Cir. 1991); M.P.E.P. § 2163.02.

Applicant submits that the specification of the captioned application provides sufficient description of *Tma* DNA polymerase mutants, strong evidence of a function/structure relationship, and adequate description of representative species. Thus, one skilled in the art could reasonably conclude that Applicant had possession of the *Tma*

DNA polymerase mutants encompassed by the rejected claims, in the present application as filed.

The level of skill and knowledge in the art on the priority date of the present application was substantial. At the time the invention was made, the sequence of many DNA polymerases had been compiled and aligned. These DNA polymerases can be divided into several distinct families based on sequence homology. Family A, or Pol I-type polymerases, exhibit significant homology to *E. coli* DNA polymerase I. Family B, or Pol II-type polymerases, exhibit significant homology to *E. coli* DNA polymerase II. Family C, or Pol III-type polymerases, exhibit significant homology to *E. coli* DNA polymerase III alpha subunit. By looking at their respective sequences, one skilled in the art would easily determine that *Tma* DNA polymerase exhibits significant homology to *E. coli* DNA polymerase I and is, thus, part of the Pol I-type family of polymerases.

In addition, the specification teaches that the *Thermotoga* DNA polymerase of the invention can be isolated from any strain of *Thermotoga* which produces a DNA polymerase. Thus, although the preferred strain to isolate the gene encoding the *Thermotoga* DNA polymerase of the present invention is *Thermotoga maritima* (*Tma*), Applicant asserts that additional DNA polymerases, including allelic variants, can be isolated from other *Thermotoga* strains. *See* specification at page 16, lines 13-24. This is easily accomplished using standard molecular biology protocols since all DNA polymerases contain several conserved regions, including the regions that contain, *inter alia*, 3' to 5' exonuclease activity and 5' to 3' exonuclease activity. *See* specification at page 18, line 26, to page 19, line 25. Mutating specific amino acid residues within these regions abolishes or reduces these

activities. *Id.* Examples of other DNA polymerases that can be so mutated are indicated on page 20, lines 8-13, of the specification.

Furthermore, by comparison of the amino acid sequence of *Tma* DNA polymerase to other DNA polymerases, consensus sequences can be identified. For example, the O-helix is a 14 amino acid sequence that when mutated renders the polymerase non-discriminating against non-natural nucleotides, such as dideoxynucleotides. *See* specification at page 21, line 21, to page 22, line 14. The consensus sequence of the O-helix may be defined as **R**XXX**K**XXX**F**XXX**Y**X, wherein X is any amino acid. *Id.* A review of the *Tma* DNA polymerase amino acid sequence, as provided in U.S. Patent No. 5,374,553 (which is incorporated by reference as indicated at page 18 of the specification), shows significant homology to the O-helix consensus sequence: amino acids 722-735 **R**RAG**K**MVN**F**SII**Y**G. Additional consensus sequences common among DNA polymerases are described below.

The preceding discussion illustrates that one skilled in the art looking at the amino acid sequence of *Tma* DNA polymerase and comparing it to other known DNA polymerases, can easily locate conserved regions and identify consensus sequences or motifs. Knowledge of these conserved regions or motifs would allow one skilled in the art of molecular biology to identify regions that can be mutated. For example, the specification discloses at page 19, lines 4-10, that the 3'→5' exonuclease activity can be reduced or impaired by creating site specific mutants within the 3'→5' exonuclease domain. In a specific embodiment of the invention Asp^{323} of *Tma* may be changed to any other amino acid, preferably to Ala to substantially reduce 3'-to-5' exonuclease activity.

The specification further discloses that the 5'→3' exonuclease activity of the *Tma* DNA polymerase can be reduced or eliminated by mutating the DNA polymerase gene by a variety of mutations including point mutations, frame shift mutations, deletions, and insertions. For example, any one of six conserved amino acids that are associated with the 5'→3' exonuclease activity can be mutated. Examples of these conserved amino acids with respect to *Tma* DNA polymerase include Asp^{8}, Glu^{112}, Asp^{114}, Asp^{115}, Asp^{137} and Asp^{139}. *See* specification at page 19, lines 11-25.

Tma DNA polymerase mutants can also be made to render the polymerase non-discriminating against non-natural nucleotides such as dideoxynucleotides. *See* specification at pages 21-22. Changes within the O-helix of *Tma* polymerase, such as other point mutations, deletions, and insertions, can be made to render the polymerase non-discriminating. *Id.* The most important amino acids in conferring discriminatory activity include Arg, Lys and Phe. Amino acids which may be substituted for Arg at positions 722 are selected independently from Asp, Glu, Ala, Val Leu, Ile, Pro, Met, Phe, Trp, Gly, Ser, Thr, Cys, Tyr, Gln, Asn, Lys and His. Amino acids that may be substituted for Phe at position 730 include Lys, Arg, His, Asp, Glu, Ala, Val, Leu, Ile, Pro, Met, Trp, Gly, Ser, Thr, Cys, Tyr, Asn or Gln. Amino acids that may be substituted for Lys at position 726 include Tyr, Arg, His, Asp, Glu, Ala, Val, Leu, Ile, Pro, Met, Trp, Gly, Ser, Thr, Cys, Phe, Asn or Gln. Most preferred mutants include Phe^{730} to Tyr^{730}, Ser^{730}, Thr^{730} and Ala^{730}. *Id.*

In addition, the specification discloses at pages 19 and 20 that to abolish 5'→3' exonuclease activity, amino acids are selected which have different properties. For example, an acidic amino acid such as Asp may be changed to a basic, neutral or polar but uncharged amino acid such as Lys, Arg, His (basic); Ala, Val, Leu, Ile, Pro, Met, Phe, Trp (neutral); or

Gly, Ser, Thr, Cys, Tyr, Asn or Gln (polar but uncharged). Glu may be changed to Asp, Ala, Val Leu, Ile, Pro, Met, Phe, Trp, Gly, Ser, Thr, Cys, Tyr, Asn or Gln.

As further evidence that the level of skill and knowledge in the art on the priority date of the present application was substantial, Applicant discusses the various known common attributes and features of *Thermotoga* DNA polymerases below, and discusses various scientific documents to strengthen and support his arguments.

The *Tma* DNA polymerases are part of the Pol I-type family of polymerases and exhibit significant homology to *E. coli* DNA polymerase I. As discussed *supra*, all DNA polymerases contain conserved regions, each of which has a consensus sequence. For example, Pol I-type DNA polymerases share many conserved sites throughout the protein. Gutman *et al.*, *Nucl. Acids Res. 21*:4406-4407 (1993) (reference AR6), describe conserved sites in the 5'-3' exonuclease domain of Pol I-type DNA polymerases. Six conserved regions, identified as regions A-F, are shown in Figure 1 of Gutman *et al.* and a consensus sequence for each of these conserved regions is provided. A review of the *Tma* DNA polymerase amino acid sequence, as provided in U.S. Patent No. 5,374,553 (which is incorporated by reference as indicated at page 18 of the specification), shows significant homology to these consensus sequences.

Similarly, Bernard *et al.*, *Cell 59*:219-228 (1989) (reference AR2), describe conserved regions in the 3'-5' exonuclease domain of Pol I-type polymerases. There is significant similarity between these conserved regions and corresponding regions in the *Tma* DNA polymerase. Additional information regarding the domain structure of *Tma* DNA polymerases, including the localization of the exonuclease activities and polymerase activity to specific domains, is available. For example, the 5'-3' exonuclease domain of *E. coli*

polymerase I is at the N-terminal end of the polymerase, while the middle and C-terminal domains of this polymerase exhibit 3'-5' exonuclease and DNA polymerase activity, respectively. *See* C. M. Joyce, *Curr. Opin. Str. Biol. 1*:123-129 (1991) (reference AS7). This domain structure is conserved in other Pol I-type polymerases, such as *Tma* DNA polymerase.

Further information regarding the domain structure of the *Tma* DNA polymerase can be seen from the functional characterization of deletion mutants of *Tne* DNA polymerase and several other Pol I-type polymerases. For example, deletion of the first 235 and 288 amino acids from the N-terminal region of *Taq* polymerase eliminates 5'-3' exonuclease activity. Similarly, deletion of the first 250 amino acids of the *Tth* polymerase and the first 323 amino acids of *E. coli* polymerase I also eliminates 5'-3' exonuclease activity. Deletion of the first 514 and 520 amino acids from the N-terminal region of *E. coli* polymerase I eliminates both 5'-3' and 3'-5' exonuclease activity.

Thus, the sequence and other information disclosed for DNA polymerases in the specification would indicate to a scientist skilled in the art that polymerase activity is localized to the C-terminal portion of the *Tma* DNA polymerase, while the 5'-3' and 3'-5' exonuclease activities are localized to the N-terminal and middle portions of this polymerase, respectively. Fragments containing the C-terminal region will typically retain polymerase activity, while fragments lacking the 5'-3' or 3'-5' exonuclease domains will not retain 5'-3' or 3'-5' exonuclease activity, respectively.

In addition, significant additional information regarding mutant forms of *Tma* DNA polymerase with reduced or no 5'-3' or 3'-5' exonuclease activity, or reduced or no discriminatory behavior against a dideoxynucleotide can be obtained when coupled with

structural information that has been obtained for *E. coli* polymerase I and other Pol I-type DNA polymerases. For example, a high resolution three-dimensional crystal structure for the Klenow fragment of *E. coli* polymerase I has been reported by Ollis *et al., Nature 313*:762-766 (1985) (reference AS7).[1] In this structure, regions that may be associated with polymerase and 3'-5' exonuclease activity are identified. Based on the sequence homology between *E. coli* polymerase I and the *Tma* polymerase, the *Tma* polymerase and *E. coli* polymerase I are expected to have a similar structure. Thus, this additional structural information provides extensive guidance to one skilled in the art to select mutants having reduced or eliminated 3'-5' exonuclease activity, while retaining polymerase activity. Based on the sequence homology between *E. coli* polymerase I and the *Tma* DNA polymerase, a scientist skilled in the art would readily be able to identify segments or specific residues of the *Tma* DNA polymerase involved in polymerase and 3'-5' or 5'-3' exonuclease activity.

Further guidance to select fragments or mutants of *Tma* DNA polymerase would be provided to a scientist skilled in the art from mutational analyses performed on *Tne* DNA polymerase and other Pol I-type DNA polymerases. Polesky *et al., J. Biol. Chem. 265*:14579-14591 (1990) (reference AR8) describe several mutations within the polymerase domain of the Klenow fragment of *E. coli* polymerase I. Mutations at residues 849, 668 and 882 show a large decrease in catalytic activity. Thus, several residues involved in polymerase activity have been identified. Each of these residues are within regions of high homology. Moreover, these residues are located near the region predicted to be associated with polymerase activity in the three-dimensional structure of the Klenow fragment. In a

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[1] The Klenow fragment of *E. coli* polymerase I also contains the 3'-5' exonuclease domain. Thus, this article also provides useful information regarding the 3'-5' exonuclease active site.

further article, several additional mutations are analyzed, confirming the location of the active site. *See* Polesky *et al., J. Biol. Chem. 267*:8417-8428 (1992) (reference AT7).

Mutational analyses have also been performed in the 3'-5' exonuclease domain of *E. coli* polymerase I. *See* Derbyshire *et al.*, *Science 240*:199-201 (1988) (reference AR3); and Derbyshire *et al.*, *EMBO J. 10*:17-24 (1990) (reference AT2). The mutations that resulted in loss of exonuclease activity, at residues 355, 424 and 501 were located in the regions of the 3'-5' exonuclease domain exhibiting a high degree of homology with *Tne* DNA polymerase and other members of the *E. coli* polymerase I family of polymerases. This information provides guidance to one skilled in the art to envision numerous specific mutations, in addition to those described in the specification, that will reduce or eliminate 3'-5' exonuclease activity.

Similarly, Pol I-type DNA polymerase mutants have been made containing mutations in the 5'-3' exonuclease domain. Several of these mutations, falling within regions of homology, reduce or eliminate 5'-3' exonuclease activity. Thus, these experiments also confirm that the selection of specific residues based on sequence homology can identify mutants expected to have reduced or eliminated 5'-3' exonuclease activity.

As discussed above, generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. In this situation: 1) there is detailed and well known structural information for DNA polymerases; 2) structure/function correlations are available for these DNA polymerases; and 3) the sequence and other structural information for the *Tma* DNA polymerases are provided in this and the priority applications. Thus, the level of

skill and knowledge in the art is substantial. Accordingly, the specificity of disclosure necessary to satisfy the written description requirement is minimal.

In conclusion, Applicant submits that: (1) the level of skill and knowledge of one skilled in the art at the time the application was filed was substantial; (2) adequate written description of a genus which embraces variant species can be achieved by disclosing only one species within the genus; (3) one skilled in the art would recognize that Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed; (4) individual support for each species that the genus embraces is not required; and (5) based on the substantial level of skill and knowledge in the art, and the fact that Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed, the disclosure in the specification necessary to satisfy the written description requirement is sufficient.

However, solely in an effort to advance prosecution, Applicant has amended claims 1 and 37 to recite that "said mutation is selected from the group consisting of: a deletion, a single or double substitution, a point mutation, a frame shift mutation and an insertion." Support for such an amendment has been described *supra* and can be found, *inter alia*, at page 18, line 26, to page 19, line 25; page 20, line 25, to page 21, line 9; and page 22, lines 12-14. Applicant submits that he has sufficiently described the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize Applicant was in possession of the claimed invention. Accordingly, withdrawal of the written description rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Other Matters

The allowance of claims 38 and 40-44 is acknowledged and appreciated by Applicant.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Gaby L. Longsworth

Gaby L. Longsworth, Ph.D.
Agent for Applicant
Registration No. 47,756

Date: January 8, 2003

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

Version with markings to show changes made

In the Claims:

The following claims 1, 9 and 37 were substituted for pending claims 1, 9 and 37:

1. (Twice amended) A *Thermotoga maritima* (*Tma*) DNA polymerase [mutant] which is modified at least two ways selected from the group consisting of:

(a) a mutation in the 3'-5' exonuclease domain of said polymerase to reduce or eliminate the 3'→5' exonuclease activity of the polymerase;

(b) a mutation in the 5'-3' exonuclease domain of said polymerase to reduce or eliminate the 5'→3' exonuclease activity of the polymerase; and

(c) a mutation in the O-helix of said polymerase to reduce or eliminate discriminatory behavior against a dideoxynucleotide;

wherein said mutation is selected from the group consisting of: a deletion, a single or double substitution, a point mutation, a frame shift mutation and an insertion.

9. (Once Amended) The mutant *Tma* DNA polymerase as claimed in claim 1, wherein [said mutant polymerase comprises both] the modifications are a $Phe^{730}\rightarrow Tyr^{730}$ substitution and an $Asp^{323}\rightarrow Ala^{323}$ substitution.

37. (Twice amended) An isolated DNA molecule encoding a *Thermotoga maritima* (*Tma*) DNA polymerase [mutant] which is modified at least two ways selected from the group consisting of:

(a) a mutation in the 3'-5' exonuclease domain to reduce or eliminate the 3'→5' exonuclease activity of the polymerase;

(b) a mutation in the 5'-3' exonuclease domain to reduce or eliminate the 5'→3' exonuclease activity of the polymerase; and

(c) a mutation in the O-helix to reduce or eliminate discriminatory behavior against a dideoxynucleotide;

wherein said mutation is selected from the group consisting of: a deletion, a single or double substitution, a point mutation, a frame shift mutation and an insertion.